

AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph on page 17, line 24 with the following amended paragraph:

The expression cassette was assembled from PCR-amplified plastid regulatory elements. The 16S rRNA promoter, PrrnL was amplified by PCR from total DNA of Nicotiana Tabacum (cv PBD6) using two specific primers:

otprnrc5: 5'-caattgtcgcgagaattcgctagcggcgccgtcccccgccgtgttc-3' (SEQ ID NO: 19)

and otprnrc3: 5'-atcgatccgcgggagctcggtaccatgcatgttagattcggaaattgtcttccctcc-3' (SEQ ID NO: 20).

Please replace the paragraph on page 17, line 35 with the following amended paragraph:

To synthesize the fusion of the 5'UTR from the G10L gene with the first 14 amino acids of the GFP (Pang et al., 1996) (G10L::14aaGFP), the following primers:

Og10L5: 5'-tatcttagaaataatttgttaactttaagaaggagatataccatggcaaggcg-3' (SEQ ID NO: 21), and

Opgfp3: 5'-ggatgcattgcttaagattgggaccacgccagtgaacagttcctgccttgccatggtatct-3' (SEQ ID NO: 22)

were annealed to each other and elongated using standard PCR technology and Pwo DNA polymerase (Roche). These oligonucleotides were also engineered in order to create a XbaI restriction site at the 5' end and BfrI and NsiI at the 3' end of the fusion G10L::14aaGFP. A NcoI restriction site is inserted at the junction between the 5'UTR of the G10L gene and the 14aa of the GFP. This NcoI site offers the possibility to eliminate the 14aaGFP if necessary. The PCR fragment was cloned in the TOPO vector (Invitrogen) to form pCLT411.

Please replace the paragraph on page 18, line 7 with the following amended paragraph:

The 2maroA gene from Salmonella typhimurium was amplified by PCR using oligonucleotides:

OaroAdb5: 5'-gcctaagctccatggaatccctgacgttacaaccc-3' (SEQ ID NO: 23), and

OaroAdb3: 5'-gcgtatgcataatttaatttagcaggcgtactcattcg-3' (SEQ ID NO: 24).

A PCR fragment was purified and cloned in the pPCRscript vector (Stratagene) to yield pCLT406.

Please replace the paragraph on page 18, line 25 with the following amended paragraph:

The two expression cassettes AADA-166 and AROA-170 were further cloned between the two recombination regions RHRR and LHRR, identical to pCLT312 either in the same or in the inverse transcriptional orientation as the native soybean 16SrDNA gene (in RRHR). In order to create appropriate restriction sites for cloning, two multiple restriction sites (SMC1 and SMC2) were obtained using standard PCR technology by annealing and elongating the following oligonucleotides OSMC5 (5'-gaaagcttcggaccgtagttaaacaggccatatggcct-3' (SEQ ID NO: 25)) with OSMC3 (5'-gactcgagttaattaatcgccgcgcaggccatatg-3' (SEQ ID NO: 26)) for SMC1 and OSMC51 (5'-gagcggccgcctcgagcggaccgtagttaaacaggccatatggcct-3' (SEQ ID NO: 27)) with OSMC31 (5'-gaaagctttaattaatcgccgcgcaggccatatg-3' (SEQ ID NO: 28)) for SMC2. The SMC1 and SMC2 were digested by HindIII and XbaI restriction enzyme and cloned into pCLT312 digested by the same enzymes to give respectively pCLT316 and pCLT315. The two expression cassettes AADA-166 and AROA-170 were cloned as a 3189 bp PmeI-PacI pCLT171 fragment into the PmeI and PacI restriction sites of pCLT315 and pCLT316 to form the plastid transformation vectors pCLT317 and pCLT318, respectively. In order to evaluate the influence of the 14aaGFP on expression of the transgene, pCLT317 and pCLT318 were digested by NcoI restriction enzyme to remove the 14aaGFP and ligated to yield pCLT319 and pCLT320, respectively. The expression cassettes of the 2maroA gene present in pCLT319 and pCLT320 are identical and are named AROA-319 (SEQ ID NO: 13). The expression cassettes are in the same transcriptional orientation as the native soybean 16SrDNA gene (RRHR) in the plasmids pCLT318 and pCLT320 or in the inverted transcriptional orientation in the plasmids pCLT317 and pCLT319.

Please replace the paragraph on page 19, line 25 with the following amended paragraph:

pCLT321 is derived from pCLT317. The NcoI/Blunt PCR heliomicin fragment amplified by PCR using the oligonucleotides P2 (5'-ACACCATGGATAAATTAAATTGG-3' (SEQ ID NO: 35)) and P3 (5'-CCTCTAGATTAAGTTCACACCAAC-3' (SEQ ID NO: 36)) from *Heliothis*

virescens genome (WO 99/53053), and recoded for expression into tobacco plastids was cloned into the NcoI and SwaI restriction sites of pCLT317, replacing the 2maroA gene. pCLT321 carries the AADA-166 and the heliomicin (HELIO-321; SEQ ID NO: 14) cassettes in the inverse transcriptional orientation as the native soybean 16SrDNA gene. The HELIO-321 cassette is driven by the PrnL fused with the RBS from the G10L but without the first 14aa of the GFP.

Please replace the paragraph on page 20, line 4 with the following amended paragraph:

The strategy for the PCR analysis of the transformants with pCLT321 was to land the primer P6 (5'-GTTAAGGTAAACGACTCGGCATGG-3' (SEQ ID NO: 39)) immediately outside the RHRR in the soybean 16SrDNA gene, outside the homologous recombination region, while landing the other one P5 (5'-ctcagtactcgagttatgcgactacccgttgcgtatctcgcc-3' (SEQ ID NO: 38)) on the aadA gene. A 2,838 bp PCR product should be obtained in the case of integration of transgene into the plastome. The expected product was observed for the transgenic calli 1, 3, and 4 obtained using the soybean vector pCLT321. Unbombarded plants (controls) did not yield any PCR products, as expected. These PCR results show that the aadA gene is really integrated into the soybean plastome at the expected locus. The integration of the two expression cassettes into the soybean plastome was demonstrated using the primers P7 (5'-CATGGGTTCTGGCAATGCAATGTG-3' (SEQ ID NO: 40)) / P8 (5'-CAGGATCGAACTCTCCATGAGATTCC-3' (SEQ ID NO: 41)) designed to land on both sides of the site of integration of the foreign gene into the LHRR and RHRR, respectively. Two 1030 bp and 3054 bp PCR products should be observed for the WT plastome and the transplastome, respectively. The expected products were obtained for the WT and the transplastomic lines 1, 3 and 4. The spectinomycin resistant lines 1, 3 and 4 are thus transplastomic. The presence of some WT fragments indicated some heteroplasmy. An additional 1666 bp PCR fragment is observed in these three transplastomic lines corresponding probably to the recombined transplastome after excision of the AADA-166 cassette by homologous recombination. The integration of the two expression cassettes into the soybean plastome was confirmed using two other sets of primers P1 (5'-CGTATCGAACATGCTTAG-3' (SEQ ID NO: 34); landing on the LHRR) /P2 (5'-ACACCATGGATAAAATTAAATTGG-3' (SEQ ID NO: 35); on the heliomicin gene) and P4 (5'-CGTCATACTTGAAGCTAGACAGGC-3' (SEQ ID NO: 37); landing on aadA) / P3 (5'-

CCTCTAGATTAAGTTCACACCAAC-3' (SEQ ID NO: 36); on the heliomicin gene). Expected PCR products of 520 bp and 922 bp corresponding to the transplastome were obtained for the transplastomic event 1, 3, and 4 using the primers P1/P2 and P4/P3, respectively.

Please replace the paragraph on page 21, line 9 with the following amended paragraph:

The hppd gene from *Pseudomonas fluorescens* (Rüetschi et al., Eur. J. Biochem., 205, 459-466, 1992, WO 96/38567) was amplified by PCR using oligonucleotides Ohppd5 (5'-gccttaagctccatggcagatctatacgaaaacccaatggc-3' (SEQ ID NO: 29)) and Ohppd3 (5'-gccatttaaattaaatcggcggtaatacaccacgacgcacctg-3' (SEQ ID NO: 30)). A 1099 bp PCR fragment was purified and cloned in the pPCRscript vector to yield pCLT409. A NcoI/SwaI pCLT409 fragment containing the hppd gene was cloned into the NcoI and SwaI restriction sites of pCLT317, resulting in pCLT323. pCLT323 carries the AADA-166 and the hppd (HPPD-323, SEQ ID NO: 15) cassettes in the inverse transcriptional orientation as the native soybean 16SrDNA gene. The HPPD-323 cassette is driven by the PrmL fused with the RBS from the G10L but without the first 14aa of the GFP.

Please replace the paragraph on page 22, line 2 with the following amended paragraph:

The cry1Ab gene from *Bacillus thuringiensis* (Bt) (GeneBank X04698) coding for the Cry1Ab protoxin was amplified by PCR using oligonucleotides OcryWT5 (5'-gccttaagctccatggataacaatccgaacatcaatg-3' (SEQ ID NO: 31)) and OcryWTL3 (5'-gccatttaaattattcctccataagaagttaattccacgtgtccacg-3' (SEQ ID NO: 32)) from *Bacillus thuringiensis* (strain berliner 1715) genome. The 5' part of the cry1Ab gene coding for the toxin was also amplified by PCR using the oligonucleotides OcryWT5 and OcryWTC3 (5'-gccatttaaattaatcatattctgcctcaaaggttacttctgcccgaac-3' (SEQ ID NO: 33)).